

1235-Plat**Force Spectrum Microscopy Reveals Active Diffusive-Like Fluctuations in Living Cells**Ming Guo¹, Allen Ehrlicher¹, Mikkel Jensen¹, Jeffrey Moore², Jennifer Lippincott-Schwartz³, Frederick Mackintosh⁴, David Weitz¹.¹Applied Physics, Harvard University, Cambridge, MA, USA, ²Boston University, Boston, MA, USA, ³NIH, Bethesda, MD, USA, ⁴VU University, Amsterdam, Netherlands.

A living cell is a far-from-equilibrium dynamic material, replete with active force generation due to molecular motors and other enzymatic activity. While motors often produce highly directed motion, the aggregate yet incoherent effect of all active processes can create randomly fluctuating forces that can lead to diffusive-like, but inherently non-thermal motion. These fluctuating forces drive many basic cellular processes, making it essential to characterize their behavior; however, there is no existing technique to measure these forces. Here we introduce a new assay to quantify the random forces directly, by combining measurements of the random motion of probe particles with independent micromechanical measurements of the cytoplasm, to quantitatively determine the spectrum of these active force fluctuations. These active forces substantially enhance intracellular movement over a broad range of length scales. Moreover, we show that this force spectrum is a sensitive assay to probe motor activity in cells: The fluctuations are three times larger in malignant cancer cells than in their benign counterparts.

1236-Plat**Myosin Light Chain Kinase Activity Regulates the Number of Leading Edges in Zebrafish Embryonic Keratocytes**

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Rapidly motile cells such as neutrophils and keratocytes typically polarize to generate a single front and rear in order to sustain productive forward movement. However, other less persistently motile cell types, such as fibroblasts and epithelial cells, frequently have multiple, apparently independent, protrusive fronts. The mechanisms that limit polarization to a single front and rear for rapidly motile cells are largely unknown. Here we use embryonic zebrafish keratocytes to investigate how a purely lamellipodial cytoskeleton self-organizes to regulate protrusion number and overall cell polarity. We have observed that keratocytes derived from 4 days post-fertilization (dpf) embryos often form multiple protrusions, suggesting that the motility machinery in these cells polarizes differently from "single front" keratocytes such as those found at 2dpf. To elucidate the mechanism for this difference in polarization behavior, we performed gene expression analysis on 2dpf and 4dpf cells and found that 4dpf cells express higher levels of MLCK. Treatment of 4dpf cells with the MLCK inhibitor ML7 can induce ~50% of multiple-front cells to form a single leading edge. To determine whether ML7 induces single-front behavior by decreasing actin turnover, we tested whether ML7 treatment synergizes with the actin filament stabilizing drug jasplakinolide, and found that >90% will form a single front in response to this drug combination. We have also found that ML7 treatment increases the width of leading edges in 4dpf cells, including those that don't form a single front. Based on these results, we speculate that increased MLCK activity in 4dpf cells increases actin turnover at the leading edge, limiting the size of each protrusion. This represents a novel mechanism for myosin activity to regulate cell polarity not by controlling the rear but rather by influencing the front from afar.

1237-Plat**Quantitative Subcellular Control of Cdc42, Rac1 and RhoA GTPases using the Cry2/CIBN Optogenetic Dimerizer**

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Cell polarization is established and maintained through complex mechanisms involving signaling networks regulated in space and time at the sub-cellular level. How a cell coordinates multiple local signaling modules to create a global polarization is still unclear. Recently developed optogenetic methods have been recognized as promising tools to dissect these intracellular signaling networks by allowing perturbations to be spatially and temporally controlled. First, we characterized the biophysical properties of the Cry2/CIBN light gated dimerization system in order to establish the quantitative relationship between patterns of light stimulation and corresponding gradients of induced signaling activity. We analyzed the processes involved in the recruitment and localization of Cry2 including the 3D cytoplasmic diffusion of Cry2, the 2D diffusion of Cry2/CIBN complexes at the cell membrane and the dissociation kinetics of this complex. From these experiments we determined that it is possible to induce subcellular gradients of recruited proteins of any chosen profile up to a spatial resolution of 5µm and a temporal one of ca. 3 minutes. Second, we applied our quantitative optogenetic method to the regulation of Cdc42, Rac1 and RhoA, the three canonical RhoGTPases involved in cell polarity

and migration. We quantified the effect of the local activation of Cdc42 using cell displacement and cell shape changes as reporters of cell polarization and migration. We qualitatively characterized the effects of local activation of RhoA and Rac1 on different cellular effectors including actin filaments and focal adhesion complexes. Altogether, our quantitative optogenetic method provides a new step for the optogenetic dissection of subcellular signaling networks by allowing the simultaneous measurement of the perturbation and the cell response in a straightforward and reproducible way.

Platform: Bioengineering**1238-Plat****Biomechanical Basis of Alzheimer's Disease and Other Protein Misfolding Diseases: Designing a New AFM Probe to Study Amyloid-Mediated Membrane Disorders**Amanda K. Woodcock¹, Brian Meckes², Ratnesh Lal².¹Bioengineering, University of Washington, Seattle, WA, USA,²Bioengineering, University of California, San Diego, La Jolla, CA, USA.

Most important biological interactions with its environment occur at interfaces such as a lipid cellular membrane. These interactions are often dynamic and show time-dependent changes in material properties (e.g. viscoelastic properties). A detailed understanding of biomechanical properties at molecular and subcellular level is vital for basic understanding of normal/abnormal biological processes. Indeed, abnormal changes in the mechanics of biological systems are often indicators of pathophysiological states. For example, amyloid beta peptide (Aβ), a protein present in neural plaques formed in Alzheimer's disease and cholesterol are reported to alter cell membrane mechanical properties leading to neuronal degeneration. Atomic force microscopy (AFM) provides an ideal tool to image the structure and examine the mechanics in physiologically relevant medium. We have created a new cantilevered probe for AFM for the study of nano-to-microscale mechanics of biological interfaces. We used it to examine the role of Aβ1-42 insertion in DPPC lipid monolayers and its effect on the viscoelastic properties that underlie altered membrane fluidity. Real-time analysis of mechanical properties of the model membrane monolayer in the presence of Aβ1-42 shows a decrease in the viscosity of the monolayers over time, consistent with increased membrane fluidity. The finding is consistent with changes in membrane permeability due to Aβ-inserted pores.

1239-Plat**Light-Powered Bionanoelectronic Devices with Biologically-Tunable Performance Characteristics**Ramya Tunuguntla¹, Kyunghoon Kim², Mangesh Bangar³, Caroline Ajo-Franklin³, Pieter Stroevé⁴, Aleksandr Noy⁵.¹Materials Science, UC Davis, Davis, CA, USA, ²Mechanical Engineering, UC Berkeley, Berkeley, CA, USA, ³Lawrence Berkeley National Laboratory, Berkeley, CA, USA, ⁴Chemical Engineering, UC Davis, Davis, CA, USA, ⁵Lawrence Livermore National Laboratory, Livermore, CA, USA.

Bacteriorhodopsin (bR) is a well-characterized photoactivated proton pump capable of creating a proton gradient using the energy of absorbed light. We are developing a bioelectronic platform that couples bacteriorhodopsin activity with the electronic response of a nanowire field-effect transistor. To accomplish this task we use hierarchical assembly of lipids and membrane proteins into a 1-D lipid bilayer structure covering the channel region of the transistor device. This presentation discusses the device preparation, characterization, and overall performance. In addition, we present results showing how other ion channels and ionophores, co-assembled with the protein embedded bilayer, can be used to change various performance characteristics of the device, independent of one-another. The ability to use components of the biological "toolkit" to fine-tune bioelectronic devices could have important implications for design of next generation biointerfaces.

1240-Plat**Light Driven Conformational Switching: An Approach to Creating Designed Protein Motion**Elizabeth Bromley¹, Lara Small¹, Asahi Cano-Marques¹, Dek Woolfson², Paul Curmi³, Martin Zuckermann⁴, Nancy Forde⁴, Gerhard Blab⁵, Heiner Linke⁶.¹Durham University, Durham, United Kingdom, ²Bristol University, Bristol, United Kingdom, ³UNSW, Sydney, Australia, ⁴SFU, Vancouver, BC, Canada, ⁵Utrecht University, Utrecht, Netherlands, ⁶Lund University, Lund, Sweden.

Biomolecular motors have inspired the design and construction of artificial nanoscale motors and machines based on nucleic acids, small molecules, and inorganic nanostructures. However, the high degree of sophistication and efficiency of biomolecular motors derives from the complexity afforded by protein building blocks. Here, we discuss a novel bottom-up approach to understanding biological motors and present a class of designs for synthetic protein motors that move along a linear DNA track.